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FACTORS INFLUENCING EMBRYONATION AND SURVIVAL OF EGGS OF THE STOMACH WORM, HAEMONCHUS CONTORTUS [1]

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INTRODUCTION

The large stomach worm, *Haemonchus contortus*, is in many respects the most serious parasite of ruminants. It occurs in the fourth stomach, or abomasum. The adult worms produce large numbers of eggs, which are in the morula stage when they are discharged with the feces of the host. Under favorable conditions the eggs develop to the tadpole stage in 8 hours or less and to the vermiform embryo stage in 24 hours. Shortly after this the eggs hatch. The first- and second-stage larvae each have a life span lasting a little more than a day under favorable conditions. The third, or infective larvae, may be produced, therefore, on the fourth or fifth day after the eggs are passed out of the host animal.

The control of these roundworms depends on a thorough knowledge of the free-living stages and of the factors that influence their development and survival. The work reported in this paper was undertaken to test the effects of certain adverse environmental conditions on the embryonation and survival of the eggs, one of the free-living stages of this parasite. The investigations were carried out at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., from August 1936 to February 1940.

MATERIALS AND METHODS

Feces of sheep experimentally infected with *Haemonchus contortus* were used as source material for the experiments. Freedom of the material from other parasites was tested by examinations of feces for eggs (*10*) [2], examination of fecal cultures for infective larvae, and post mortem examinations of the gastrointestinal tracts of some of the host animals that had served as a source of eggs. The only other nematode found was *Strongyloides papillosus*. For purposes of the present investigation, the presence of *S. papillosus* was not a complicating factor because this nematode is readily differentiated in all its stages from the corresponding stages of *H. contortus*.

Unless otherwise stated, all cultures were made from 24-hour fecal samples collected in muslin bags attached to rams or wethers and, therefore, not contaminated with urine. The fecal pellets were pulverized and the resulting mass thoroughly mixed, because it had been previously determined that more larvae developed in feces treated in this manner than when the unbroken pellets were cultured.

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[2] Numbers in parentheses refer to Literature Cited, p. 286.

Cultures were first exposed to conditions differing from those previously determined as optimum for development and were returned to optimum conditions for a sufficient time to permit the surviving eggs and larvae to develop to the infective stage. Conditions were considered to be optimum (1) when the material in the culture had the same moisture content as the fecal pellets in their original form; (2) when the cultures were kept in the dark, the culture dish being so placed that free circulation of air was possible; and (3) when the temperature was maintained at 70° to 80° F.

After incubation, the cultures were placed in the Baermann apparatus with water at 104° F. Twenty-four hours later the first 30 cc. of water was drawn off and the larvae counted. When only a small number of larvae were recovered they were counted directly; when large numbers were present a dilution method of counting was used. With the above method, from 50 to 80 percent of the eggs originally found in control cultures were recovered as larvae. Egg counts were made by the Stoll technique.

Control cultures were made from weighed samples of feces and were handled in the same manner as the test cultures, except that they were not exposed to adverse conditions.

EXPERIMENTAL DATA

OXYGEN REQUIREMENTS

Parona and Grassi (*7*) noticed that feces in a fluid (liquid or diarrheic) state were not a good culture medium for the growth of larvae of the hookworm of man. Perroncito (*8*) verified this observation and found that it applied also to cultures of *Strongyloides*. Boycott and Haldane (*2*) found that immersion in water stopped the development of eggs or larvae of *Ancylostoma duodenale* and eventually killed them. Veglia (*14*) reported similar findings for the eggs and larvae of *Haemonchus contortus* and also stated that development failed to take place in wet or immersed feces as the eggs were killed unless water was removed in less than 7 days. When the water was more than 0.5 inch deep the larvae usually died while in the first stage. This inhibition of development of eggs and larvae in wet or immersed feces has also been found by a number of investigators for other nematodes, as follows: By Theiler and Robertson (*13*) for *Ornithostrongylus douglasi* (Cobbold, 1882) Travassos, 1920 (syn. *Trichostrongylus douglasi* (Cobbold, 1882) Theiler and Robertson, 1915); by Leiper (*4*) for helminths producing thin-shelled eggs; by Lerche (*5*) for *Syngamus trachea*; by Mönnig (*6*) for *Trichostrongylus* spp., *Haemonchus contortus*, and *Oesophagostomum columbianum*; by Poluszynski (*9*) for horse strongyles; by Spindler (*12*) for *Stephanurus dentatus*; and by Skriabine and Shultz (*11*) for *Dictyocaulus* spp.

In the present experiment cultures made in half-gallon jars always yielded fewer larvae than cultures made in pint jars, when feces from the same source and in equal quantities were used in both types of jars and also when there was more material in the larger jars. Indications are that the higher survival of larvae in the pint jars was due to a better diffusion of gases formed in the cultures, but this point was not determined.

When free circulation of air was prevented in cultures by sealing the jars, no further development took place, although the control

cultures with air admitted freely produced an abundance of larvae. In the sealed jars the available oxygen apparently was rapidly used up by the bacteria present in the feces. Since merely sealing a culture jar was found to prevent further development, the question arose as to the fate of the eggs under such conditions. In order to answer this question, 21 cultures, each containing 50 gm. of feces, were prepared in fruit jars. Nineteen of these jars were sealed and air was admitted freely to the other two. Fresh air was admitted to one of the sealed cultures at the end of 24 hours and to the remainder at varying intervals by removing the rubber gasket and lid and allowing a current of air from an electric fan to blow across the opening of the jar for about a minute before the lid was replaced. Of the cultures in sealed jars, 15 were kept for 6 days at room temperature (75° to 80° F.) after the fresh air was admitted and then examined for infective larvae. The remaining 4 sealed cultures were opened and examined immediately to determine whether any development of larvae had taken place while they were sealed.

As shown in table 1, no larvae were recovered from any of the cultures examined immediately after air was admitted. In the cultures examined 6 days after air was admitted, the results showed that prevention of free circulation of air for 1 day may have been sufficient to destroy the vitality of a large number of the eggs, but that it took more than 3 weeks but less than 4 weeks to devitalize all eggs. The obvious conclusion is that after 21 days all available oxygen was used up or the carbon dioxide became so concentrated that the eggs or larvae died.

TABLE 1.—*Effect of exclusion of oxygen on survival of eggs of Haemonchus contortus in culture* [1]

Culture No.	Time air was excluded from culture	Infective larvae recovered from cultures examined—		Culture No.	Time air was excluded from culture	Infective larvae recovered from cultures examined—	
		6 days after air was admitted	Immediately after air was admitted			6 days after air was admitted	Immediately after air was admitted
	Days	*Number*	*Number*		*Days*	*Number*	*Number*
1	1	18,960		11	27	0	
2	4	5,600		12	27		0
3	7	2,400		13	37	0	
4	7		0	14	37	0	
5	8	1,904		15	37	0	
6	13	6,148		16	37	0	
7	15	3,552		17	37	0	
8	15		0	18	37	0	
9	21	71		19	37	0	
10	21		0				

[1] Two unsealed control cultures yielded 28,350 and 52,500 larvae.

This experiment showed that the eggs remained viable long after there was insufficient oxygen left for embryonation to proceed. In this connection, Boughton and Hardy (*1, p. 218*) reported that "*H. contortus* larvae, grown in sheep manure in an air-tight mason jar for nine months in the dark at room temperature, were viable and infective when administered orally to a parasite-free lamb." Since no details are given by these authors, it is impossible to state whether the feces placed in air-tight mason jars contained eggs or infective

larvae, but in view of the results of the present experiment it appears probable that infective larvae rather than eggs were present.

In view of the fact that there is a marked reduction in the number of larvae developing in cultures under reduced oxygen conditions, it appears that only a few larvae of *H. contortus* would develop in sheep feces dropped or washed into ponds or other bodies of water.

EFFECT OF HIGH AND LOW TEMPERATURES

Veglia (*14*) reported that *Haemonchus contortus* eggs embryonated normally at temperatures between 68° and 95° F., which he considered to be optimum. The following experiments were performed to determine the effect on the eggs of exposure to less favorable temperatures.

In these experiments pint-jar cultures, after they had been exposed to unfavorable temperatures in incubators and electric refrigerators, were maintained at room temperature for a period sufficient to allow

TABLE 2.—*Effect of exposure to low and high temperatures on survival of eggs of Haemonchus contortus*

Experiment No.	Temperature	Exposed culture No.	Time of exposure	Infective larvae recovered from—	
				Exposed cultures	Control cultures[1]
	°F.		Days	Number	Average number
1	10	1	1	2	7,562
		2	2	0	
		3	3	0	
2	15	1	2	280	3,781
		2	3	0	
3	25	1	2	14	14,310
		2	2	44	
4	33	1	1	9,280	40,260
5	33	1	2	330	14,310
		2	2	400	
		1	1	1,222	
		2	2	928	
		3	3	370	
6	33–39	4	4	39	7,562
		5	5	39	
		6	6	63	
		7	9	0	
7	36	1	1	1,234	3,781
		2	2	84	
8	37–45	1	2	3,478	7,562
		2	3	1,404	
9	39–49	1	14	24	10,710
		2	28	0	
10	40	1	1	33,840	40,260
11	40–52	1	14	63	10,710
		2	28	1	
12	45–55	1	14	123	10,710
		2	28	11	
13	90–96	1	2	5,160	7,562
		2	3	7,380	
		3	4	9,480	
		4	5	8,900	
14	93–99	1	2	5,000	7,562
		2	3	9,890	
		3	4	8,940	
		4	5	8,020	
15	98	1	1	2,320	3,781
		2	2	1,634	
16	98	1	1	7,875	14,310
		2	2	2,400	
			Hours		
17	140	1	0.5	5,560	10,710
		2	1	2,244	
		3	1.5	1	
		4	2	2	
		5	2.5	0	
		6	3	0	

[1] Exposed at room temperature—about 70° F.

any surviving eggs to hatch and for the larvae to reach the infective stage. The results of 17 experiments are shown in table 2.

The data in this table show that any marked deviation from the temperatures found by Veglia to be optimum caused a drop in the number of larvae reaching the infective stage or of eggs capable of reaching the infective stage when subjected to favorable conditions. These data also give some new information on the rapidity of devitalization of eggs of *H. contortus*, namely, that few eggs remained viable for 1 or 2 days at temperatures below freezing (experiments 1, 2, and 3), and that in 1-day exposures to temperatures just above freezing (experiments 4, 6, and 7) two-thirds to five-sixths of the eggs became devitalized. The data are especially important in showing that few eggs of *H. contortus* are able to survive as long as 2 weeks at temperatures only 12° to 28° F. below those considered optimum for embryonation (experiments, 9, 11, and 12).

Veglia (*14*) found that some second-stage larvae developed from eggs kept at temperatures as low as 46.4° F. Observations not included in table 2 indicated that no development of larvae to infectivity took place in cultures kept at temperatures below 55°, although a few eggs survived at these temperatures for as long as 4 weeks, as shown in experiments 11 and 12 of table 2. From this information, as well as from the data shown in table 2 that only 2 to 12 out of 1,000 eggs survived temperatures of 39° to 55° for 2 weeks, and only about 1 out of 1,000 survived at 55° for 4 weeks, it may be inferred that *H. contortus* eggs in feces deposited on pasture will not yield larvae capable of reaching infectivity while the weather is cold.

To determine the effect of outdoor temperatures during December on the survival of eggs of *H. contortus*, nine 4-inch Petri-dish cultures each containing 20 gm. of feces, were prepared. Seven of these were placed outside in an experimental plot, and two were retained in the laboratory as controls. The cultures placed outdoors were covered with opaque paper and were removed to the laboratory one at a time. During the 9 days that the cultures were outdoors the temperature fell below freezing four times, once on each of the first 4 days of the experiment. The lowest temperature recorded, 17° F., occurred during the second night of the experiment. All cultures were examined 6 days after the last culture had been brought into the laboratory. The results are recorded in table 3.

TABLE 3.—*Survival of eggs of Haemonchus contortus exposed to outdoor temperatures in December*

Culture No.	Outdoor temperatures		Time of exposure	Total period each culture was below freezing	Larvae recovered [1]
	Minimum	Maximum			
	°F.	°F.	Days	Hours	Number
1	28	37	1	6	46
2	17	39	2	22	0
3	17	50	3	36	10
4	17	60	5	46	0
5	17	63	7	46	4
6	17	63	8	46	0
7	17	63	9	46	0
8 [1]					120
9 [1]					220

[1] Control cultures.

The glass of the culture dishes and the opaque paper protected the eggs in the cultures to some extent, yet the table shows that none of the eggs were able to survive after the seventh day of exposure. In the greater part of the sheep-raising areas of the United States the temperature for half the year or more is usually too low for survival of eggs of this parasite (*3*).

EFFECTS OF DRYING

A review of the literature indicates that eggs of *Haemonchus contortus* (*6*, *14*) and those of other bursate nematodes in freshly passed feces are destroyed by complete drying. Four experiments were performed (table 4) to determine the degree of dryness required to kill these eggs. In all tests dry, pulverized feces were used, and in one (the third) the pulverized feces were dried until they could be sifted through a 40-mesh screen. Feces were weighed before and after drying, and distilled water in an amount equal to that lost was added. After the water was restored, the cultures were kept about 1 week at room temperature to allow any surviving eggs to hatch and the larvae to reach infectivity.

TABLE 4.—*Survival of eggs of Haemonchus contortus in cultures dried to temperatures of 70° to 80° F.*

Experiment No.	Exposed culture No.	Time of exposure to drying	Relative humidity	Temperature	Larvae recovered from—	
					Dried cultures	Control cultures
		Hours	*Percent*	°*F.*	*Number*	*Number*
1	1	7	[1] 25	70	84	162,019
2	1	7	70–80	80	83,790	[2] 704,035
	2	16			269,990	
3	1	4	50–53	80	102	[3] 7,425
	2	22			4	
	3	30			0	
	4	48			0	
	5	54			0	
4	1	3	53	80	1,000	165,000

[1] Calculated from outdoor humidity.
[2] Average of 2 cultures.
[3] Average of 5 cultures.

The first experiment was made while the building was heated and the relative humidity was, accordingly, low. No humidity readings were taken, but as the outdoor relative humidity was 49 percent at 47° F., the calculated indoor relative humidity, if condensation and some other factors are ignored, was about 25 percent. The second experiment was performed during the summer when the temperature was rather high. During the time of drying culture No. 1, the air was circulated by a fan, but culture No. 2 was dried overnight, beginning at 4 p. m., and moistened the following day at 8:30 a. m.; the fan was not operating while the latter culture was drying. At the time the eggs were exposed to drying a larger proportion of those in culture 2 than in culture 1 were in the vermiform stage.

The third and fourth experiments were made in the fall; a fan was used to hasten drying. The only difference between the five cultures used in the third experiment was the number of hours the cultures were exposed to drying. The first three experiments were carried

out with a 24-hour fecal collection of eggs, which, therefore, were in the morula, tadpole, and vermiform stages. The fourth experiment involved a 2-hour fecal collection of eggs, of which 88 percent were in the morula stage and the others in the early tadpole stage.

The fact that eggs in the vermiform stage are resistant to drying (*14*) may account for some of the difference in the number of larvae recovered from the cultures. However, these tests show that eggs in all stages of embryonation are susceptible to the effects of desiccation. Decrease in viability appears to be due to the completeness of the drying of feces regardless of whether lower relative humidity or air currents are involved in the drying process.

As shown by the table, all the above-mentioned tests were carried out at room temperature (70° to 80° F.). Other tests were carried out at higher and lower temperatures to determine the effect on egg viability of differences in temperature during the drying process. For these tests eight 20-gm. fecal cultures were prepared in 4-inch Petri dishes and dried at four different temperatures. The cultures were paired, one being allowed to dry by leaving it uncovered while the other was kept covered and served as a control. When the cultures were returned to room temperature after 48 hours, all uncovered cultures except one at 34° appeared to be dry. The cultures were weighed, the moisture lost was replaced with distilled water, and the cultures kept for 15 days before being examined. The results are shown in table 5.

TABLE 5.—*Survival of eggs of Haemonchus contortus in cultures subjected to drying at temperatures of 10° to 97° F.*

Experiment No.	Temperature	Larvae recovered from—	
		Dried cultures	Control cultures
	°F.	*Number*	*Number*
1	10	1	0
2	34	1,190	407
3	70 to 80	0	10,902
4	97	0	6,800

When drying and cold were applied simultaneously to eggs of *H. contortus*, the effect of one moderated the effect of the other, as shown by the data in table 5 considered in conjunction with data in tables 2, 3, and 4. On the other hand, when heat and drying were employed together, the effect on the eggs was intensified as is also shown by the data in table 5, together with those of tables 2 and 4, probably because drying is more rapid at higher temperatures.

DISCUSSION

The experiments reported in this paper show that the eggs of *Haemonchus contortus* in fresh feces are deleteriously affected by drying, lack of oxygen, heat, and cold. From the data it may be inferred that in the western half of the United States, where two-thirds of the sheep and lambs are raised, the climate is usually too dry in the summer and too cold in the winter for the survival of eggs of *H. contortus*, except on irrigated pastures. Nearly all the remaining third

of the sheep and lambs raised in this country are found where the winters are cold and embryonation of eggs in normal years would be possible only from May to September, inclusive. Although the experiments involving the effect of reduced oxygen supply on the survival of *H. contortus* eggs may be only of theoretical importance so far as field conditions are concerned, they indicate that as a source of *Haemonchus* infection swamps and ponds with much organic matter may have been overrated. Most *H. contortus* eggs falling in such ponds or on swampy ground would die for lack of sufficient oxygen.

SUMMARY AND CONCLUSIONS

To test the effect of lack of oxygen, drying, and adverse temperatures on the survival of eggs of the large stomach worm, *Haemonchus contortus*, investigations were carried out at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., from August 1936 to February 1940. Feces of sheep experimentally infected with *H. contortus* were used as source material for the experiments.

After culture jars were sealed so that no fresh air could enter, embryonation of *H. contortus* eggs in the cultures ceased, and in less than 4 weeks but more than 3 weeks the viability of the eggs was destroyed.

At temperatures below 55° F. no development to infectivity of *H. contortus* took place, and death resulted if the exposure was sufficiently long. Constant temperatures above 98° were deleterious to the eggs. There was little difference in the number of larvae that developed from eggs kept at temperatures of 90° to 96° and 93° to 99° and those kept at 70°. The lethal effect observed increased with the deviation from the optimum temperatures.

Drying killed eggs rapidly, but a few survived several hours in apparently dry feces. When the moisture content of the feces was lowered by drying the feces at a relatively low humidity or by means of air currents at a relatively high humidity, the death rate of eggs was raised.

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STUDIES ON THE USE OF THE POINT-QUADRAT METHOD OF BOTANICAL ANALYSIS OF MIXED PASTURE VEGETATION [1]

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INTRODUCTION

Since the development of the point-quadrat method for the analysis of pasture vegetation originated by Levy and Madden [3] in New Zealand, there have appeared in the United States a number of studies on its use and applications. Following an intensive comparative investigation in Wisconsin of several methods of botanical analysis, Tinney, Aamodt, and Ahlgren [4] concluded that the point-quadrat method gave the greatest promise of yielding reliable data consistent with rapidity and ease of operation. Hanson [5] had earlier found that the method was reliable when employed in analytical work on the native prairies of western North Dakota. More recently, Arny and Schmid,[6] working with various pasture mixtures in Minnesota, have investigated critically the use of the inclined point-quadrat method. These investigators showed that in mixtures of tall legumes and fine-leaved grasses correction factors must be computed in order precisely to evaluate the data, since the broad-leaved legumes tended to be hit less per gram of dry weight than the narrow-leaved grasses.

The present study was undertaken in order to determine (1) the relative merits of the point-quadrat method as compared with the count-list method for sampling small areas of a low-growing, grass-lespedeza pasture; and (2) the relative efficiency of four different point-quadrat methods for the quantitative analysis of such vegetation.

The investigation was carried out during July and August 1942 on a lespedeza-small grain rotation pasture in the vicinity of Columbia, Mo. Because of unfavorable planting conditions in the fall of 1941, it was impossible to sow the small grain successfully. Hence, the next spring and summer the pasture had an astonishingly uniform cover of the palatable fall panicum, (*Panicum dichotomiflorum*), which had replaced the grain, in addition to an excellent stand of lespedeza (*Lespedeza stipulacea*). While the fall panicum is hardly in the usual

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category of forage crops, in its growth form under pasture conditions it closely resembles several domesticated grasses. Thus the results reported herein are thought to be reasonably applicable to lespedeza—grass mixtures in which the grass component is broader-leaved than Kentucky bluegrass.

PRELIMINARY COMPARISON OF THE POINT-QUADRAT, COUNT-LIST, AND WEIGHT-LIST METHODS OF ANALYSIS

PROCEDURE

An area supporting a relatively uniform mixed stand of grass and lespedeza, and having practically uniform soil and topographic conditions, was selected, and a plot of 100 m.[2] (10×10 m.) was staked off. Ten one-half-square-meter quadrats were then selected at random. In all cases, the order of study was as follows: (1) Count-list; (2) inclined point-quadrat; (3) straight point-quadrat; (4) weight-list. After careful counting of stems of individual plants [7] with a minimum of disturbance of their natural positions of growth, the point-quadrat apparatus was set up parallel to the long axis and across the half-square-meter sample area. Readings were first taken with the apparatus set up in the inclined (45°) position; and, then, without moving the two front supports, the apparatus was so placed that the needles were vertical. In each position, two types of data were recorded as follows: (1) The first species hit per needle (method A); and (2) the total number of hits obtained by pushing each needle to the ground, irrespective of whether the same plant or different ones were hit once or more than once (method B). Second and third sets of readings of 10 needles each were next obtained by resetting the apparatus in the quadrat, diagonally to the right, and then diagonally to the left. Afterwards, the entire one-half-square-meter area was clipped by hand at the soil surface, and, later, the species were hand-separated in the laboratory, the vegetation was oven-dried, and dry weights were obtained. For convenience, three components of the vegetation were separated, as follows: (1) Lespedeza; (2) grasses; (3) forbs.[8] Since other legumes were very rare, and since 98 percent of all grasses was fall panicum, no further separation appeared necessary. This part of the study was carried on only in early July.

RESULTS

The data in table 1 summarize the results of the count-list determinations. Although variation from quadrat to quadrat is evident, standard deviations and standard errors were not computed because the comparative magnitude of differences between methods is such as to indicate quite clearly these essential differences.

Comparing the percentage composition figures of table 1 with those of table 2, it is obvious that the use of the count-list method results in a decided underestimate of the amount of vegetation furnished by lespedeza, and vice versa, a noticeable overestimate of the grass

[7] In the case of grasses, culms were taken as the unit for counting.

[8] The forbs included all broad-leaved herbs that were samples. The following species were the principal ones sampled: Bracted plantain (*Plantago aristata*), bullnettle (*Solanum carolinense*), cocklebur (*Xanthium* sp.), common ragweed (*Ambrosia artemisiifolia* var. *elatior*), curly dock (*Rumex crispus*), field dodder (*Cuscuta pentagona*), knotweed (*Polygonum aviculare*), lanceleaf ragweed (*Ambrosia bidentata*), milk purslane (*Euphorbia supina*), Pennsylvania smartweed (*Polygonum pensylvanicum*), peppergrass (*Lepidium virginicum*), red sorrel (*Rumex acetosella*), spiny sida (*Sida spinosa*), yarrow (*Achillea millefolium*).

element. Forbs were relatively scarce, and are not important enough to be worth further consideration at this time. So far as lespedeza is concerned, an undercount was certainly to be expected because of the widely branching nature of the plant. Similarly, the overcount of grasses further emphasizes the difficulties inherent in any direct counting of stems of individuals of such plants. The unit used for direct counting was the culm; but the point-quadrat data for grasses include hits on any aerial part of the plant.

TABLE 1.—*Summary of results of count-list determinations*

Quadrat No.	Lespedeza		Grasses		Forbs	
	Plants	Composition	Plants	Composition	Plants	Composition
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	564	38.6	886	60.6	11	0.8
2	313	20.7	1,115	73.6	87	5.7
3	388	31.7	821	67.0	16	1.3
4	283	22.9	920	74.3	35	2.8
5	364	30.8	748	63.2	71	6.0
6	287	29.3	670	68.4	23	2.3
7	367	42.9	461	53.9	27	3.2
8	294	26.4	771	72.3	15	1.3
9	269	27.2	645	70.3	25	2.5
10	291	28.8	625	69.9	13	1.3
Total	3,420	299.3	7,662	673.5	123	27.2
Mean	342	29.9	766.2	67.4	32.3	2.7

TABLE 2.—*Summary of results of weight-list determinations*

Quadrat No.	Lespedeza		Grasses		Forbs	
	Weight	Composition	Weight	Composition	Weight	Composition
	Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
1	28.05	57.3	20.05	41.0	0.85	1.7
2	20.25	39.2	28.05	54.4	3.3	6.4
3	24.2	48.6	24.15	48.5	1.45	2.9
4	18.7	36.5	29.9	58.3	2.7	5.2
5	29.3	54.3	22.9	42.4	1.8	3.3
6	20.5	48.6	20.3	48.1	1.4	3.3
7	24.3	60.5	14.0	34.8	1.9	4.7
8	24.3	37.1	39.4	60.2	1.8	2.7
9	17.1	35.7	28.7	59.9	2.1	4.4
10	17.0	43.6	20.7	53.1	1.3	3.3
Total	223.7	461.4	248.15	500.07	18.6	37.9
Mean	22.37	46.1	24.815	50.01	1.86	3.79

An examination of the data obtained by using the point-quadrat methods (table 3) discloses that, even when a minimum of 10 needles per half-square-meter quadrat is used, the percentage composition of the elements of the vegetation quite closely approaches that obtained from dry weights. So far as the four methods of employing the apparatus are concerned, the data are not sufficiently extensive, nor have they been sufficiently tested, to permit the drawing of inferences. Yet it is fairly clear that somewhat greater accuracy—assuming that the data obtained from dry weights provide a satisfactory basis for comparison—is obtained by the inclined method, where the needles are pushed to the ground and all hits tabulated (method B). Moreover, from these preliminary trials, it is also evident that the fore-

going statement holds, though with some variation, for all the elements of the vegetation. No well-marked tendencies toward underhitting of legumes or overhitting of grasses are ascertainable. The addition of further readings from a second and third set within the half-square-meter area merely resulted in slight percentage changes, the net result of the total hits of 30 needles per quadrat being nearly the same as that from 10 needles. From these preliminary studies, and under the conditions described, it would appear that for sampling small areas a large number of readings is not necessary.

TABLE 3.—*Summary of data obtained by point-quadrat methods* [1]

HITS ON LESPEDEZA

Quadrat No.	Needles straight				Needles inclined 45°			
	A		B		A		B	
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1	4	40	21	45.7	[2] 4	40	[2] 17	40.5
2	5	50	[2] 25	59.5	[2] 6	60	[2] 13	44.8
3	2	22.2	6	18.8	4	40	12	33.3
4	4	40	22	61.1	[2] 4	44.4	[2] 21	51.2
5	4	44.4	15	57.7	6	60	24	70.6
6	5	50	13	38.2	2	20	12	35.3
7	0	0	5	21.7	[2] 2	22.2	[2] 22	48.9
8	[2] 4	44.4	[2] 12	63.2	[2] 1	10	[2] 15	40.5
9	[2] 2	20	[2] 8	25	[2] 4	40	[2] 12	26.7
10	5	55.6	14	46.7	[2] 4	44.4	[2] 17	45.9
Total	35		141		37		165	
Mean of 10 quadrats	3.5	36.7	14.1	43.7	3.7	38.1	16.5	43.8
Mean of 20 quadrats	3.8	41.0	13.8	46.5	3.6	37.0	15.4	48.0
Mean of 30 quadrats	3.9	39.1	14.4	45.7	3.6	36.4	15.5	41.3

HITS ON GRASSES

1	6	60	25	54.4	[2] 5	50	[2] 23	54.8
2	5	50	[2] 15	35.7	[2] 3	30	[2] 15	51.7
3	7	77.8	26	81.3	6	60	24	66.6
4	6	60	14	38.9	[2] 4	44.4	[2] 19	46.3
5	5	55.6	11	42.3	4	40	10	29.4
6	5	50	31	61.8	8	80	22	64.7
7	9	100	18	78.3	[2] 5	55.6	[2] 18	40
8	[2] 4	44.4	[2] 6	31.6	[2] 8	80	[2] 20	54.1
9	[2] 7	70	[2] 23	71.9	[2] 6	60	[2] 32	71.1
10	4	44.4	16	53.3	[2] 4	44.4	[2] 18	48.6
Total	58		175		53		201	
Mean of 10 quadrats	5.8	61.2	17.5	54.9	5.3	54.4	20.1	52.7
Mean of 20 quadrats	5.2	57.4	15.2	52.1	5.5	57.3	19.1	53.5
Mean of 30 quadrats	5.3	57.0	16.6	53.3	5.6	57.8	20.5	55.0

HITS ON ALL FORBS

Mean of 10 quadrats	0.2	2.1	0.4	1.3	0.7	7.2	1.4	3.7
Mean of 20 quadrats	.1		.4		.4		1.1	
Mean of 30 quadrats	.1		.3		.6		1.4	

[1] In this and subsequent tables A is used to indicate only the first hit on vegetation tabulated; B to indicate all hits on vegetation tabulated, as needle is pushed to the ground, irrespective of whether a given plant is hit once or oftener.

[2] One or more hits on forbs were obtained in this quadrat. Although not listed separately in a table because of their relative unimportance to the study as a whole, the hits on forbs were included in calculating percentage composition of lespedeza and grasses. These small percentages of forbs therefore account for the apparent discrepancies in the composition figures for lespedeza and grasses.

COMPARISON OF DIFFERENT METHODS OF USING THE POINT-QUADRAT APPARATUS FOR BOTANICAL ANALYSIS

PROCEDURE

In larger areas of the same pasture that was described in the first section of this report, a more extensive investigation was directed at comparing the 4 different applications of the point-quadrat method to which reference has already been made. Ten sample areas had been selected previously and covered with wire cages (approximately 4×4 feet) to exclude grazing animals. During early July, and again in August, the point-quadrat apparatus was set up at random in 2 separate places within the sample area. Readings were first taken with the needles inclined at an angle of 45°, and 2 sets of data, as before, were recorded. Without moving the front supports, the needles were then set vertically, and a second set of data was obtained. Following the completion of such readings, a rectangular area (6×19 inches), equivalent to that covered by vegetation through which the inclined needles passed, was carefully clipped, and the species later separated by hand. This procedure was repeated for the other set on the same plot, and then for each of the remaining 9 similar plots. A total of 200 needles for each of the 4 methods was thus obtained.

In August after the vegetation had had a month of undisturbed growth, the foregoing procedure was repeated for each sample area, except that the parts previously harvested were avoided in making the tests. By August the vegetation had attained a fairly uniform height of 6 inches, whereas in July the general level had been 4 inches. Since the vegetation was 2 inches taller in August, a slightly larger rectangular area (8 × 19 inches) was harvested for dry-weight determinations. By using such small harvested areas it was hoped that the correlation of percentage composition between the oven-dry weight, for a given element of the vegetation, and that determined by point-quadrat methods would be more precise, a relationship well demonstrated by the work of Arny and Schmid.[9] Furthermore, if greater precision were thus attained, the relative merits of the four methods tested would be thrown into sharper contrast and be of proportionately greater significance.

RESULTS

The data given in tables 4 and 5 summarize for lespedeza the results for July and August obtained by using the 4 techniques available with the point-quadrat method. Number of hits and percentage composition per quadrat (set of 10 needles) for lespedeza are here indicated. While the mean percentage composition contributed by lespedeza in July varies considerably with the method used, the standard deviation and standard errors clearly indicate that a more reliable result is obtained by recording all hits (method B), irrespective of whether the needles are vertical or inclined at an angle of 45°. Moreover, by comparing with these results the figures for July on percentage composition of lespedeza on a dry-weight basis (table 4) the greater reliability of this method (B) of counting all hits is further substantiated. The data for July appear to indicate that somewhat more satisfactory results can be obtained with the inclined method

[9] See footnote 6.

than with the vertical one, but the August data fail to confirm this finding. It should be recalled, however, that the vegetation in August was a good 2 inches taller, suggesting the possibility that in a vertical position the taller, flat-bladed lespedeza might have been hit more often than if the needles had been inclined.

TABLE 4.—*Summary of data obtained in point-quadrat determinations for lespedeza in July*

Quadrat No.	Needles straight				Needles inclined 45°				Lespedeza composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Precent*
1	[1]3	33.3	[1]11	31.4	4	40	[1]18	51.4	8.2	46.1
2	[1]6	60	[1]22	62.8	4	40	[1]27	56.2	11.5	63.8
3	[1]3	30	[1]21	52	4	40	[1]27	60	5.3	43.3
4	5	50	16	57.1	5	50	20	57.1	6.6	50.9
5	5	50	15	46.9	4	40	27	56.2	6.8	51.9
6	[1]5	55.6	[1]13	44.8	[1]6	54.5	[1]24	55.8	6.5	55.56
7	7	70	[1]25	73.5	7	70	22	53.6	7.5	63.8
8	5	50	22	61.1	7	70	22	59.4	9.3	76.3
9	6	60	23	69.7	8	80	33	70.2	7.4	72.3
10	6	60	27	65.8	6	60	21	55.2	6.9	62.8
11	7	77.8	15	55.6	8	80	33	78.6	7.0	71.6
12	6	60	29	69.05	8	80	33	73.3	9.4	82.3
13	[1]5	50	[1]21	60.0	[1]5	50	[1]20	54	6.8	56.7
14	8	80	23	62.2	8	80	30	71.4	9.0	73.6
15	6	60	18	54.5	4	40	35	72.9	4.0	68.4
16	7	70	[1]36	85.7	[1]7	70	[1]38	80.8	9.3	71.7
17	[1]3	30	[1]18	47.4	[1]4	40	[1]21	48.8	7.7	60.6
18	3	30	21	47.7	[1]4	40	[1]34	68	9.8	64.6
19	6	60	27	69.2	5	50	28	70	11.8	75.4
20	7	70	[1]24	60	[1]3	30	[1]22	50	5.0	51.9
Total	109		427		111		535		158.8	
Mean	5.5	58.6	21.4	58.9	5.6	55.5	26.8	62.1	7.9	64.1
Standard deviation		15.24		12.19		16.55		9.96		9.77
Standard error		3.41		2.70		3.70		2.23		2.18

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 3.

The data for the grasses are given in tables 6 and 7. As in the case of lespedeza, these results indicate that for the grasses involved the counting of the total number of hits (method B) is more accurate than merely listing the first species hit per needle (method A). Standard deviations and standard errors both bear out this assertion. Moreover, the inclined method, under the conditions prevailing in July appeared to give slightly better estimates for the percentage composition furnished by grasses, as judged from the data for dry weight of grasses, given in table 6. The figures for August parallel those for lespedeza in showing that the most reliable results were obtained by counting the total hits when the needles were in a vertical position, but the differences obtained by this method and that in which the needles are inclined were slight. Both methods, as compared with dry-weight percentage figures, gave reliable results.

TABLE 5.—*Summary of data obtained in point-quadrat determinations for lespedeza in August*

Quadrat No.	Needles straight				Needles inclined 45°				Lespedeza composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	4	40	15	40.5	[1] 2	20	[1] 14	31.8	5.6	34.8
2	2	20	29	49.2	2	20	22	37.9	6.0	36.6
3	3	30	32	61.5	5	50	34	52.3	8.6	50.4
4	6	60	22	53.3	2	20	22	35.5	8.8	47.8
5	4	40	38	64.4	5	50	43	55.1	15.4	67.5
6	6	60	38	58.5	6	60	[1] 44	61.1	12.4	59.9
7	7	70	55	76.4	8	80	45	73.8	15.9	69.7
8	6	60	49	70	3	30	37	62.7	12.5	62.2
9	4	40	32	64	4	40	48	68.6	14.3	67.8
10	6	60	28	73.1	6	60	46	61.3	12.5	63.4
11	8	80	48	81.4	7	70	56	81.2	18.2	84.2
12	7	70	39	65	1	10	45	57.7	11.2	54.3
13	5	50	31	55.5	6	60	40	72.7	11.7	64.8
14	4	40	30	50	3	30	31	50	9.7	47.8
15	4	40	39	63.9	3	30	33	60	11.0	56.4
16	6	60	37	67.3	2	20	38	52.0	14.6	58.6
17	5	50	43	68.3	5	50	40	72.7	11.5	65.3
18	5	50	60	77.9	7	70	60	73.2	14.7	68.1
19	5	50	47	70.1	3	30	38	57.6	11.4	57.9
20	4	40	45	70.3	5	50	51	73.9	10.5	61.4
Total	101		777		85		787		236.5	
Mean	5.1	50.5	35.9	64.2	4.3	42.5	39.4	60.2	11.8	59.0
Standard deviation		14.68		10.32		20.23		13.64		11.35
Standard error		3.28		2.31		4.52		3.05		2.53

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 3.

TABLE 6.—*Summary of data obtained in point-quadrat determinations for grasses in July*

Quadrat No.	Needles, straight				Needles, inclined 45°				Grass composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	[1] 5	55.5	[1] 23	65.7	6	60	[1] 15	42.8	8.7	48.8
2	[1] 3	30	[1] 12	34.2	6	60	[1] 20	41.7	6.5	36.0
3	[1] 5	50	[1] 17	42	6	60	[1] 16	35.5	4.4	44.2
4	5	50	12	42.8	5	50	15	42.8	4.4	39.9
5	5	50	17	53.1	6	60	21	43.8	6.2	47.3
6	[1] 2	22.2	[1] 13	44.8	[1] 4	36.4	[1] 18	41.8	4.9	41.9
7	3	30	[1] 8	23.5	3	30	19	46.3	4.1	34.9
8	5	50	14	38.9	3	30	15	40.6	2.8	21.0
9	4	40	10	30.3	2	20	14	29.8	2.8	27.3
10	4	40	14	34.1	4	40	17	34.7	3.9	36.1
11	2	22.2	12	44.4	2	20	9	21.4	2.7	27.6
12	4	40	13	30.95	2	20	12	26.7	2.0	17.5
13	[1] 3	30	[1] 7	20.0	[1] 2	20	[1] 7	18.9	2.1	17.5
14	2	20	14	37.8	2	20	12	28.6	3.1	25.3
15	4	40	15	45.5	6	60	18	27.1	3.1	30.3
16	3	30	[1] 5	11.9	[1] 2	20	[1] 7	14.9	3.2	24.7
17	[1] 6	60	[1] 17	44.7	[1] 5	50	[1] 21	48.8	4.9	38.6
18	7	70	23	52.3	[1] 5	50	[1] 15	30	5.2	34.2
19	4	40	12	30.8	5	50	12	30	3.8	24.3
20	3	30	[1] 15	37.5	[1] 6	60	[1] 21	47.7	4.6	47.8
Total	79		273		82		299		83.4	
Mean	3.95	40.0	13.7	38.3	4.1	40.3	14.95	34.7	4.2	33.4
Standard deviation		13.52		12.21		16.86		9.99		9.82
Standard error		3.02		2.73		3.77		2.23		2.19

[1] 1 or more hits on forbs were obtained in this quadrat. See footnote 2 of table 8.

TABLE 7.—*Summary of data obtained in point-quadrat determinations for grasses in August*

Quadrat No.	Needles, straight				Needles, inclined 45°				Grass composition (dry-weight basis)	
	A		B		A		B			
	Hits	Composition	Hits	Composition	Hits	Composition	Hits	Composition		
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Grams*	*Percent*
1	6	60	22	59.5	[1] 7	70	[1] 27	61.4	10.3	64.0
2	8	80	30	50.8	8	80	36	62.1	9.5	61.1
3	7	70	20	38.5	5	50	31	47.7	8.4	49.2
4	4	40	28	46.7	8	80	40	64.5	9.6	52.1
5	6	60	21	35.6	5	50	35	44.9	6.6	28.9
6	4	40	27	41.5	4	40	[1] 27	37.5	7.0	33.8
7	3	30	17	23.6	2	20	16	26.2	6.9	30.2
8	4	40	21	30	7	70	22	37.3	7.6	37.8
9	6	60	18	36	6	60	22	31.4	6.8	32.2
10	4	40	14	26.9	4	40	29	38.7	7.2	36.5
11	2	20	11	18.6	3	30	13	18.8	3.4	15.8
12	3	30	21	35	9	90	33	42.3	9.4	45.6
13	5	50	22	41.5	4	40	15	27.3	6.2	34.3
14	6	60	30	50	7	70	31	50	10.5	51.8
15	6	60	22	36.1	7	70	22	40	8.5	43.6
16	4	40	18	32.7	8	80	35	48.0	10.3	41.4
17	5	50	20	31.7	5	50	15	27.3	6.1	34.7
18	5	50	17	22.1	3	30	22	26.8	6.9	31.9
19	5	50	20	29.9	7	70	28	42.4	8.3	42.1
20	6	60	19	29.7	5	50	18	26.1	6.6	38.6
Total	95		418		114		517		156.1	
Mean	4.8	50.0	20.9	35.8	5.7	57.0	25.9	40.0	7.8	40.3
Standard deviation		14.68		10.32		19.76		13.03		11.45
Standard error		3.28		2.31		4.42		2.91		2.56

[1] 1 or more hits on forbs were obtained in this quadrat. See footnotes of table 3.

From the data given in tables 4, 5, 6, and 7, it is clear that, at least for the heights of vegetation considered, there is no necessity for the computation of correction factors. These might be required, however, for a finer-leaved grass element.

Although some forbs were sampled and their percentage composition calculated as for lespedeza and the grasses, the mean percentage, as determined on a dry-weight basis, was 2.4 for July and 0.67 for August. Therefore it does not seem necessary to consider them in detail. In general, it might be remarked that the percentage composition of the vegetation furnished by forbs was more accurately sampled by the inclined-point method wherein all hits were tabulated.

A consideration of the factors of time and ease of operation of the various methods was outside the immediate objective of this study. Yet, as shown by other investigators, including Tinney, Aamodt, and Ahlgren,[10] the use of the point-quadrat method, regardless of what special application may be made of the apparatus for a particular investigation, is definitely time- and labor-saving as compared with most other methods of botanical analysis. So far as counting all hits versus counting only the first plant hit per needle is concerned, the latter method is undoubtedly the more rapid; but, as indicated by the general results of this study, greater accuracy is obtained by counting all hits per needle as the latter is pushed into the soil. The

[10] See footnote 4.

particular method to be employed will depend to a considerable extent on the degree of accuracy required in a given investigation.

SUMMARY

During July and August 1942 a study of the uses of the point-quadrat method of botanical analysis of a lespedeza-grass pasture mixture was undertaken (1) to determine the relative merits of this general method as compared with those of the count-list method, and (2) to ascertain the relative efficiency of four different applications of the point-quadrat method, for the same vegetation.

In a study made on one-half-square-meter quadrats, the results, though not subjected to statistical analysis, indicate that, for the vegetation analyzed, all four applications of the point-quadrat method yield more satisfactory results than the count-list method, assuming that weight-list data furnish a satisfactory basis of comparison. Moreover, no tendencies toward underhitting of the legume or overhitting of the grass were indicated in the results so that evaluation of correction factors do not appear necessary.

Results obtained from studies carried out in July and August, suggest that on the low-growing vegetation of the tests, the counting of all hits as the needles of the point-quadrat apparatus are pushed through the plants to the ground yields more reliable results than merely recording the first plant hit by each of the 10 needles. For the lower-growing (about 4 inches tall) vegetation in July, the inclined-point method gave more satisfactory results, as judged from calculated standard errors, than the straight method. Yet, in August, with the vegetation some 2 inches taller, the reverse appeared to be true. Height of the vegetation, as well as the morphology of the species involved, thus appeared to influence the manner of the use and the evaluation of results obtained by means of the point-quadrat method. Thus, it is likely that the most satisfactory method of botanical analysis will vary with the type of vegetation, and that this method should be evaluated on the basis of repeated trials.

REGRESSION OF INSECT DENSITY ON DISTANCE FROM CENTER OF DISPERSION AS SHOWN BY A STUDY OF THE SMALLER EUROPEAN ELM BARK BEETLE[1]

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INTRODUCTION

In a study of the smaller European elm bark beetle (*Scolytus multistriatus* (Marsham)) in its role as a vector of the Dutch elm disease, the writers have secured data showing distribution patterns around centers of dispersion. The results are of practical importance from the standpoint of control in indicating distance of spread and any directional tendencies that may appear. They also give an idea of the form of regression of population density on distance, a subject that is of considerable interest in insect ecology. Critical statistical methods are needed for such a study. Records of insect dispersion occur frequently in entomological literature, but usually without sufficient quantitative information for analysis. "Dispersion" as the term is here used includes active or passive movement resulting in distribution and has a broader meaning than that given to "migration" by Williams (*13*).[3] The subject has recently been reviewed by Whitfield (*12*).

In dispersion radiating from a center several elements will contribute to the observed change in insect density with distance. In the first place density will grow less as the circumference expands. This by itself should give a density having a positive linear relation to the inverse of distance, and its relation to distance itself would be of hyperbolic form. Secondly, the trend will be modified by various factors, chiefly the tendency of insects to stop on finding satisfactory places. This tendency will cut down the absolute numbers, probably rapidly at first and more slowly later. Finally, the density will often fall, not to zero, but to some low level characteristic of the general region. Centers of positive attraction, or a marked directional reaction, may obscure this picture, but in many cases it will probably hold. Observation of some effect of the insects' activities may serve almost as well as direct counts to evaluate density.

In studying records in the literature on various insects, as well as the bark beetle data, the writers decided not to attempt at first to fit a curve adapted to the three elements named. The second element seemed too uncertain in its action and in its relation to the first. Precise data in sufficient quantity were lacking. Distance must evidently have a twofold effect, but the inverse of the square of the distance did not show a usable relation. An empirical study of trend seemed best at first.

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[3] Numbers in parentheses refer to Literature Cited, p. 307.

Preliminary plotting suggested curves of gently declining slope. Later the simple semilogarithmic curves were used, and more complex curves were tried. In most cases the form of the data determines at once which of the equations $Y=a+b \log X$ or $\log Y=a+bX$, where X is distance and Y is density, should be used. Curves of the type used in this study can apparently be fitted fairly well by either (*4*). Frampton, Linn, and Hansing (*5*), dealing with a plant-disease spread by insects, used logarithms of density and related this curve form to that of diffusion. Their article contains a detailed mathematical development. Their case differs somewhat from the one studied by the writers in that it deals with infiltration from a large area rather than dispersion from a small center.

When logarithms of distance are used, no reasonable interpretation can be made at zero distance, and the curve reaches zero density at some finite distance. When logarithms of density are used, no such zero density is reached. These are trivial objections in a preliminary study of trends.

TENDENCIES SHOWN BY SOME PREVIOUS DISPERSION RECORDS

In dispersion data on codling moth adults given by Van Leeuwen (*11*), density showed a fairly close linear relationship to the logarithm of distance. Steiner (*10*) showed a somewhat similar relationship in the dispersion of the newly hatched larvae. Gaines (*6*) studied boll weevil activity as related to distance from hibernation quarters, McColloch (*8*) studied dispersion of the hessian fly, and Eckert (*3*) studied flight distances of bees. Other data on dispersion are given by Hawley (*7*) on Japanese beetle larvae, and by Wolfenbarger (*14*) on potato flea beetles as measured by larval injury. Annand, Chamberlin, Henderson, and Waters (*1*) showed the decrease in incidence of curly top in beans as an index of beet leafhopper abundance.

The graphs of all these records showed similar trends. There was a steep falling off from the center, or point of origin. As the distance increased, the slope became less steep although tending toward a low level. When the data were plotted on semilogarithmic paper, the trend approximated a straight line. The logarithmic plotting, however, did not always give a straight line all the way, as the curve often flattened out when a low level was reached. This flattening may perhaps be related to the general population level characteristic of the region. Often only a few points were available from which to plot the curve; therefore, the data were frequently not suitable for intensive study. In some of these cases the conditions were similar to those studied by Frampton, Linn, and Hansing; some were typically dispersions from a center.

AREAS UNDER OBSERVATION

In the authors' work on *Scolytus multistriatus* exceptional opportunities to observe dispersion from a center were found in two locations. In both places considerable emergence from a known center occurred. The index of activity used was the feeding in twig crotches by newly emerged adults. Only injuries that could obviously have been made by adults from the observed source were counted.

Crotch feeding has been observed to be a better criterion of general dispersion than the formation of egg galleries. The latter activity

is strongly influenced by the attractiveness of suitable trees or parts of trees, but crotch feeding by new adults seems to occur rather universally in elms. The uneven distribution of elms in the terrain caused some irregularity in the data, as isolated elms standing in open spaces were attacked more than others. However, the most important features of dispersion were sufficiently marked to appear even with this irregularity.

The first location was in a wide valley in northern New Jersey where abundant elm slash had been left in a small area. *Scolytus multistriatus* had bred in enormous numbers, and twig injury was unusually abundant. The country was rather open, with scattered trees, elms being fairly numerous. Examination of 70 trees 5 inches or more in diameter was made at definite distances in 5 directions from the center to 1,200 feet; 1 tree was examined at 1,300 feet and 3 more at 2,600. Distances were measured by pacing from the edge of the small area of heavy breeding. The trees were sampled by examining branches taken from the upper part of the crown. On each branch taken, all twig-crotch injuries of the year of exceptional beetle flight were counted. Usually 100 or more crotches were examined per tree. The type of injury and the method of examination and determination of year of injury have been described by Wolfenbarger and Buchanan (*15*). In this area the injury, although several years old when examined late in 1938, could be determined with certainty. The use of the percentage of crotches attacked in each tree as an index of activity compensates for the unequal size of the samples and seems the best measure of effect. Exclusion of small trees helps to make the data comparable.

The second location was in southwestern Connecticut. The terrain was hilly, with woodland to the west and farms with scattered trees to the east. The beetles had been active the previous year. Early in 1939 examinations were made on 108 trees 4 inches or more in diameter, in several directions, as in the New Jersey area. A number of trees were selected up to 3,000 feet from the center and some others from 5,000 to 7,200 feet. Several hundred crotches were examined in every tree, and as many as 2,000 in distant trees very lightly attacked. *Scolytus multistriatus* was not present in appreciable numbers except in the center of dispersion, as determined by intensive study of the location and general examination of the surrounding area.

RESULTS IN THE NEW JERSEY AREA

In the New Jersey area a comparatively rapid rate of decrease in the number of injured twig crotches was observed as the distance from the center increased. At the center an average of nearly 40 percent of the tree crotches were injured and all trees were heavily attacked. When a distance of over 600 feet had been reached, however, the average was only 2 to 4 percent, and little further decrease in the proportion of injured crotches was noted. There was considerable variation among individual trees. The highest percentage from a tree between 600 and 1,000 feet from the center was 8.7. At distances of 1,000 to 1,300 feet 15 trees were examined; in one, at 1,025 feet, the injury was 18 percent; in another, at 1,180, it was 12 percent; no other injury higher than 5 percent was recorded. These high individual values may have been due to favorable location or attractiveness or to secondary centers. The few trees at 2,600 feet showed about 3 percent

attack. In table 1 are brought together averages from 70 trees, arranged by directions and distance groups. From 1 to 7 trees are represented by each entry in the table.

TABLE 1.—*Percentages of crotches per tree injured by Scolytus multistriatus, classified according to distance and direction from the source, New Jersey area*

Distance (feet)	Northwest	West	Southeast	Northeast	Southwest	Average[1]
0–50	36.4	35.8	29.9	33.8	36.4	35.5
51–200	18.7	14.0	30.2	----------	9.6	17.3
201–400	9.4	6.1	20.3	----------	----------	12.4
401–600	11.0	2.8	16.2	----------	1.0	10.4
601–800	1.2	2.5	2.2	----------	2.3	2.1
801–1,000	1.6	1.3	4.3	0.9	----------	2.7
1,001–1,200	----------	2.0	2.1	8.1	2.2	3.8

[1] Averages weighted by the number of trees in each distance and direction class.

To test the significance of the dispersional differences between distances and between directions, the data were statistically examined by the method of analysis of variance (table 2). As many of the percentages were less than 10, they were converted into equivalent angles according to the method described by Bliss (*2*). A special treatment for uneven frequencies (*9, Sec. 11.9*) was adopted. The fact that not all directions are represented in all distance classes makes analysis difficult. The apparent importance of distance makes it necessary to allow for it in any analysis of direction. The percentages of injured crotches for four distance classes (0–50, 51–200, 401–600, and 601–800 feet) and four directions (northwest, west, southwest, and southeast) were used for analysis, because for these classes data were available for all co binations of direction and distance. In these data 35 trees were represented and the larger part of the important observations were included. The class at 201–400 feet, which was omitted, contained only 6 trees, and the northeast class ,0–50 feet, only 2; these appeared to show no tendencies differing much from those shown by the other classes and directions under observation. It was necessary to use a few classes with only 1 tree each, but this is not believed to have biased the error estimate.

TABLE 2.—*Analysis of variance of angles corresponding to percentages of crotches attacked, New Jersey area*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between directions	3	123.77	41.26
Between distances	3	1,779.17	593.06
Interaction	9	231.81	25.76
Error	19	----------	19.57

Distance shows a strong, clear-cut influence, but there is little evidence of the influence of direction. According to table 1, the southeast direction showed, on an average, higher percentages of crotches attacked, but the individual tree injuries in this direction varied considerably. When distance classes including all trees out to 1,000 feet from the center were analyzed without regard to direction, highly significant differences were found. Beyond about 600 feet no significant difference among distance classes could be demonstrated.

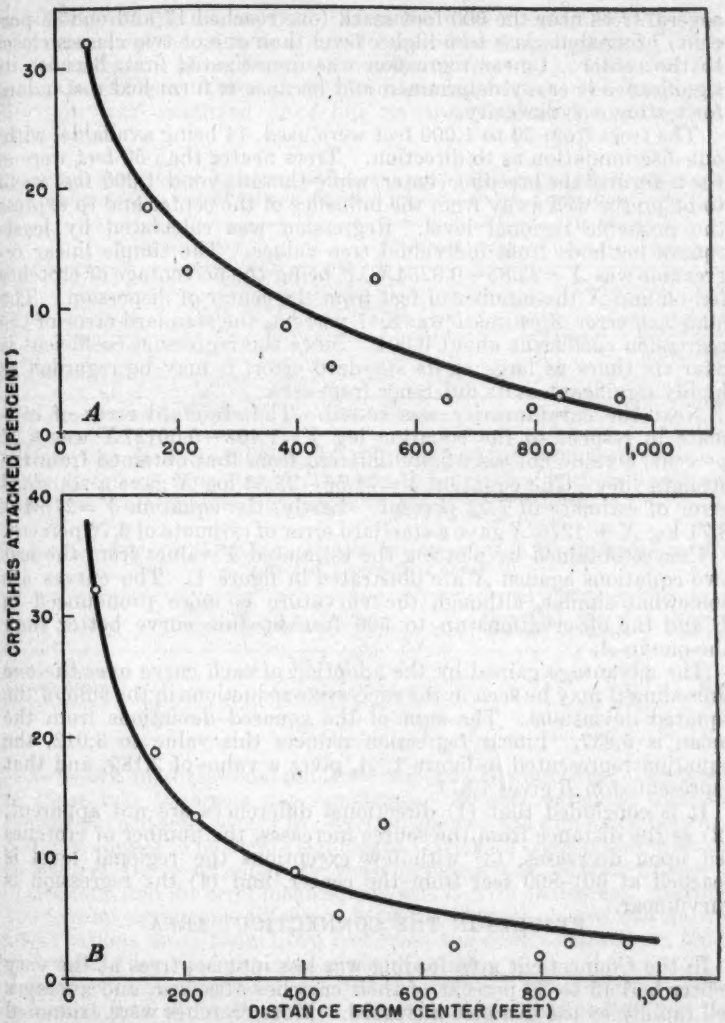

FIGURE 1.—Regression of percentage of crotches fed upon by *Scolytus multistriatus* on distance from breeding center, New Jersey area. *A*, *Y* values obtained from the equation $Y=71.56-23.55 \log X$, plotted against *X*; *B*, *Y* values from the equation $Y=27.94-8.71 \log X+1276/X$, plotted against *X*. The small circles are group means for trees 50–100 feet from the center and then at 100-foot intervals out to 1,000 feet.

It is true that distance classes could differ without showing a real regression. Preliminary plotting of individual percentages against distance indicated a definite slope, with a strong suggestion of curvilinearity, but much individual variation. The high percentages in

several trees near the 600-foot mark (one reached 17 and one 33 percent) bring that class to a higher level than one or two classes closer to the center. Linear regression was investigated first, because its significance is easily determined and because it furnished a standard for testing curvilinearity.

The trees from 50 to 1,000 feet were used, 44 being available, without discrimination as to direction. Trees nearer than 50 feet were in the margin of the breeding center, while those beyond 1,000 feet seem to be pretty well away from the influence of the center and to express the probable regional level. Regression was calculated by least-square methods from individual tree values. The simple linear regression was $Y=23.85-0.0254X$, Y being the percentage of crotches fed on and X the number of feet from the center of dispersion. The standard error of estimate was 8.47 percent, the standard error of the regression coefficient about 0.004. Since the regression coefficient is over six times as large as its standard error, it may be regarded as highly significant in its difference from zero.

Next the curvilinearity was tested. The standard error of estimate in respect to the equation $\log Y=1.408-0.00127X$ was 8.27 percent, a value not essentially different from that obtained from the straight line. The equation $Y=71.56-23.55 \log X$ gave a standard error of estimate of 7.22 percent. Lastly, the equation $Y=27.94-8.71 \log X+1276/X$ gave a standard error of estimate of 6.76 percent.

Curves obtained by plotting the estimated Y values from the last two equations against X are illustrated in figure 1. The curves are somewhat similar, although the curvature is more pronounced in *B* and the observations up to 500 feet fit this curve better than the one in *A*.

The advantage gained by the adoption of each curve over the one preceding it may be seen in the successive reductions in the sum of the squared deviations. The sum of the squared deviations from the mean is 5,837. Linear regression reduces this value to 3,012, the equation represented in figure 1, *A*, gives a value of 2,189, and that represented in *B* gives 1,871.

It is concluded that (1) directional differences are not apparent, (2) as the distance from the source increases, the number of crotches fed upon decreases, (3) with few exceptions the regional level is reached at 601–800 feet from the center, and (4) the regression is curvilinear.

RESULTS IN THE CONNECTICUT AREA

In the Connecticut area feeding was less intense; trees at the very center had 15 to 20 percent of their crotches attacked, and averages fell rapidly as the distance increased. More crotches were examined per tree than in New Jersey. Of 26 trees between 400 and 1,000 feet from the center, 1 had 5.8 percent of the crotches attacked (890 feet), 1 had 1.6 percent (470 feet), and 1 had 1.2 (671 feet); all the others had less than 1 percent. Of 30 trees between 1,000 and 2,000 feet from the center, only 4 had 1 percent or more of the crotches attacked, the maximum being 3 percent. Over 2,000 feet from the center 20 trees were examined. None had as many as 1 percent of the crotches attacked, but about half showed some injury. The most distant tree (7,180 feet) showed 0.2 percent of the crotches attacked. If,

as is believed, the beetles from the center were almost the only ones in the area, spread of over a mile by individuals is suggested.

Class averages of percentages from the 58 trees up to 1,000 feet are shown in table 3. Only three directions are shown; the southeast direction was examined also, but no trees occurred nearer than 1,000 feet.

TABLE 3.—*Percentages of crotches per tree injured by Scolytus multistriatus, classified according to distance and direction from the source, Connecticut area*

Distance (feet)	Northwest	Southwest	Northeast	Average [1]
0–50			10.41	10.41
51–100	4.18	5.30	6.19	5.44
101–200	2.60	3.18	1.29	2.40
201–400		1.56		1.56
401–600	.20	1.09		.44
601–800	.32	.18		.18
801–1,000	1.01			1.01

[1] Averages weighted by the number of trees in each distance and direction class.

Only two distance classes, 51–100 feet and 101–200 feet, have all three directions represented; 17 trees are included in these 6 groups, and these were analyzed as before (table 4).

TABLE 4.—*Analysis of variance of angles corresponding to percentages of crotches attacked, Connecticut area*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between directions	2	10.26	5.13
Between distances	1	28.56	28.56
Interaction	2	7.90	3.95
Error	11		13.55

As before, no directional influence is seen. The influence of distance in these two classes is not significant. A more extended analysis carried out without regard to direction showed the difference between distance classes to be highly significant. With 43 trees at distances up to 600 feet, the mean square between distances was 166 with 6 degrees of freedom, and the error mean square was 25 with 36 degrees. Beyond 400 feet no significant difference between distance classes was shown. Observations more than 1,000 feet from the center showed no tendencies differing from the ones already noted.

Regression equations were calculated as before. Preliminary plotting indicated that the regression of percentages of crotches fed upon per tree on distance from the center was similar to that obtained with the New Jersey material. The linear regression coefficient was -0.0124 ± 0.0029 percent. The reality of a negative regression is thus clearly shown. Observations from 42 trees within the range of 7 to 600 feet furnished the data for the analyses. The following equations, together with their standard errors of estimate, were obtained:

Equation	Standard error of estimate
$Y = 6.31 - 0.0124\ X$	3.65 percent
$Y = 18.19 - 6.77 \log X$	2.96 percent
$Y = 10.77 - 3.84 \log X + 69/X$	2.74 percent

The curve obtained by plotting the estimated Y values from the last equation against X is illustrated in figure 2. Residual sums of squares are as follows: From mean, 733; from straight line, 533; from semilogarithmic curve, 349; from modified semi-logarithmic curve, 292. Again significant improvement is shown in each step.

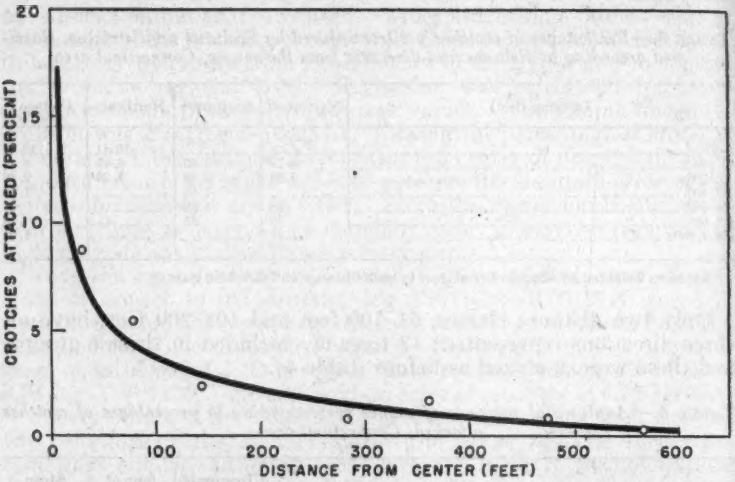

FIGURE 2.—Regression of percentage of crotches fed upon by *Scolytus multistriatus* on distance from breeding center, Connecticut area. Y values obtained from the equation $Y=10.77-3.84 \log X + 69/X$, plotted against X. The small circles are group means for trees at 7–50 and 51–100 feet from the center and then at 100-foot intervals to 600 feet.

Since it appeared that the beetles from the center under consideration were practically the only ones in the area, there was little question of a regional level. Hence curve fitting might have been extended farther from the center, probably with increased advantage for the curve over the straight line. However, such extension would lead to inclusion of a number of very low percentages and seemed unlikely to show any tendencies not already brought out.

DISCUSSION

Because of the consistency in the observations in New Jersey and Connecticut, we can regard the curvilinear relation between insect density and distance as established, and the curve of the equation $Y=a+b \log X+c/X$ as very promising for representing the relation. Several other curves allowing twice for effect of distance were tried with the New Jersey and Connecticut data, but none gave such good results as the modified semilogarithmic curve. This curve, however, cannot be regarded as precisely adapted to the factors affecting density. If allowance is made for increase in periphery, the numbers reaching various distances will form a frequency distribution that increases at first and then decreases.

SUMMARY

Following a consideration of the factors that may govern curves of insect dispersion, two pronounced infestations of *Scolytus multistriatus* (Marsham), one in New Jersey and the other in Connecticut, were studied, with twig feeding by adults as a measure of insect density. In neither infestation could indications of directional influence be found. In each case, however, pronounced falling off in density with increasing distance from the center was noted, the downward slope of the plotted curve tending to level off as the distance increased. At 401–800 feet from the center a low level of population was reached and farther out decline was hardly detectable, except for a few individual trees. The high level of general activity around the center extended only a few hundred feet. Indications were that individual beetles might travel more than a mile. In the range from the center to the zone where leveling off was marked, the decline showed in each location an undoubted curvilinear regression, although with variable material and moderate numbers its advantage over linear regression was not pronounced. Best results in fitting were given by the curve of the equation $Y=a+b \log X+c/X$, in which X is distance and Y the percentage of crotches attacked.

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